User Guide

# SMC® Human PD-1 High Sensitivity Immunoassay Kit

## Microparticle Assay

Human PD-1 Immunoassay Kit for the Quantitative Determination of PD-1 in Human Serum and Plasma

**03-0207-00**





00100402 Rev 10/25

# Introduction

Programmed cell death protein 1 (PD-1), also known as CD279, is a type I membrane protein of 288 amino acids found in T cells, B cells, and macrophages that binds Programmed cell death ligand 1 and 2 (PD-L1 and PD-L2). It is found in a soluble form and an insoluble form. PD-1 regulates the body's immune response through a variety of pathways, down-regulating immune system activity and suppressing T-cell inflammatory activity. PD-1 has been implicated as playing a role in cancers, autoimmune diseases, the immune response to viruses, and in immunological pathways related to brain repair. As an immune checkpoint, PD-1 promotes apoptosis of antigen-specific T-cells in lymph nodes and reduces apoptosis in regulatory T-cells. Overexpression of PD-1 in CD8+ T cells indicates T-cell exhaustion. In monocytes, the binding of PD-L1 to PD-1 induces IL-10 production, inhibiting CD4 T-Cell function. These activities prevent autoimmune diseases but also prevent the destruction of cancer cells by the immune system. Blockers of the PD-1 pathway have been approved as cancer therapeutics.

The SMC® Human PD-1 High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure PD-1 in Human Serum and Plasma samples. A capture antibody specific for Human PD-1 has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the PD-1 present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to PD-1 that has been captured onto the beads, thus completing the sandwich. Elution buffer is added to dissociate the protein sandwich, releasing the fluor-labeled antibodies. The eluted antibodies are transferred to a SMCxPRO® 384-well Read Plate. The plate is loaded into the SMCxPRO® or FemtoQuest™ System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of PD-1 present in the sample. The amount of PD-1 in unknown samples is interpolated from a standard curve.

# Supplies

The SMC® Human PD-1 High Sensitivity Immunoassay Kit includes all reagents listed below; these components are lot matched and not intended to be used separately. Additional reagents and supplies are required to run this immunoassay, as listed in the next section, Additional Supplies Required (Not provided).

This kit and all reagents supplied are for research use only.

## Reagents Included with the Kit

All items are shipped with a cold pack unless otherwise stated.

| Description | Storage Conditions | Packaging Details | Component Number |
|---|---|---|---|
| Assay Buffer | 2-8 °C | 2 x 20 mL | 02-9953-00 |
| PD-1 Coated Beads | 2-8 °C | 1 x 550 µL | 02-2207-00 |
| Standard Diluent | 2-8 °C | 2 x 20 mL | 02-0225-02 |
| PD-1 Detection Antibody | 2-8 °C | 1 x 270 µL | 02-1207-00 |
| PD-1 Standard | 2-8 °C | 1 lyophilized vial | 02-8207-00 |
| 10X Wash Buffer | 2-8 °C | 3 x 50 mL | 02-0001-03 |
| Buffer D | 2-8 °C | 1 x 6 mL | 02-0446-00 |
| Elution Buffer B | 2-8 °C | 1 x 5 mL | 02-0211-02 |
| SMC® Commercial Plate | 2-8 °C | 1 plate | 02-1PCP-00 |

## Kit Storage

The SMC® Human PD-1 High Sensitivity Immunoassay Kit should be stored at 2-8 °C. Discard standards after one use.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup® Filter, for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X wash buffer should be discarded upon experiment completion.

Proper kit performance can only be guaranteed if the materials are stored properly.

## Additional Supplies Required (Not provided)

Catalogue numbers are provided to purchase products at SigmaAldrich.com or through sales quote, unless otherwise noted.

**Equipment**

- SMCxPRO® Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- FemtoQuest™ System for sample acquisition (95-0200-00)
- Orbital microplate shaker for assay plate incubation (for example, Boekel Scientific Jitterbug™ Shaker)
- BioTek® 405™ TSUV Plate Washer for SMC®/MILLIPLEX® Technology (95-0004-06)
- Sphere Mag Plate for performing microparticle capture (90-0003-02)
- Rotisserie tube rotator for microparticle suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x *g* for sample/plate centrifugation
- Microcentrifuge capable of reaching 13,000 x *g* for reagent/sample centrifugation
- Single channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- 12-channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

**Supplies**

- Micro-centrifuge tubes for sample preparation and storage
- 1 L Container with cap for Wash Buffer dilution
- Stericup® Quick Release Vacuum Filtration System, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- MultiScreen®$_{HTS}$ 96-Well Filter Plate, hydrophilic PVDF membrane (MSBVN1210)
- 15 mL conical tube with cap for capture bead and detection antibody dilution
- 96-well V-bottom plate for assay setup (AXYP96450VCS)
- Axygen™ Microplate Sealing Film and Tapes (Fisher Scientific, 14-222-344)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- 12-Channel reagent reservoir (sterile) for standard serial dilution (Argos/Cole Parmer, 04395-33)
- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Millex® Syringe Filter, 0.2 µm for detection antibody filtration (SLGPR33RS)
- Luer-Lok® Syringe, 5 mL; for Detection Antibody Filtration (Fisher Scientific, 14-829-45)
- Nunc™ Aluminum adhesive plate seals (Fisher Scientific, 276014)

**Reagents**

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-00)
- De-ionized or distilled water for dilution of 10X Wash Buffer

# Assay Best Practices

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. In addition, proper training as well as instrument maintenance is critical for obtaining optimal results in performing SMC® assays. The following notes should be reviewed and understood before the assay is set up.

- Wipe down bench and pipettes with 70% isopropanol before use.
- It is important to allow all reagents to warm to room temperature (RT), 20-25 °C.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation.

  **Note:** It is recommended that the standards are prepared as the last step prior to plate setup.
- All washing must be performed with the Wash Buffer provided.
- An orbital microplate shaker for assay plate incubation (example, Boekel Scientific Jitterbug™ Shaker settings #3-5) provide maximal orbital mixing without splashing liquid or causing cross-contamination.
    - Jitterbug™ Shaker setting #3 ~ 750 rpm
    - Jitterbug™ Shaker setting #4 ~ 875 rpm
    - Jitterbug™ Shaker setting #5 ~ 1000 rpm

  **Note:** If using different orbital shaker, refer to recommended rpm ranges provided for each incubation step, and adjust speeds as necessary to ensure maximal orbital mixing without splashing liquid or causing cross-contamination.
- As the SMC® assay is extremely sensitive to dust particles, do not perform the assay or plate washing under direct airflow.
- Plate must also be protected from light after adding detection.
- After the assay is complete, seal the plate before reading immediately or storing temporarily at 2-8 °C. The SMCxPRO® and FemtoQuest™ Systems require the use of aluminum adhesive plate seal.
- It is not recommended to store eluted products from SMC® assays overnight at 4 °C or frozen at –80 °C for later reading as performance cannot be guaranteed.
- If SMC® Read Plate has been stored at 4 °C, plate should be left at RT for 30 minutes to 1 hour on the benchtop before reading to avoid a rapid increase in temperature within SMC® Read Plate wells. Bring to RT then centrifuge the plate at 1,100 x *g* for 1 minute prior to reading.
- For optimal SMCxPRO® Immunoassay System performance, perform ASSIST testing daily (ideally at beginning of the day before assay is prepared).
- For optimal FemtoQuest™ System performance, perform Self-Test daily and SMC® Fluorescence Verification Kit monthly.

# Precautions

Use caution when handling biological samples. Wear protective clothing and gloves. Components of this reagent kit contain Sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing Sodium azide should be disposed of properly.

## Hazard Labels

| Ingredient | Cat. No. | Label | |
|---|---|---|---|
| PD-1 Standard | 02-8207-00 |  | **Warning.** Harmful if swallowed, in contact with skin or if inhaled. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Wash contaminated clothing before reuse. Dispose of contents/ container to an approved waste disposal plant. |
| PD-1 Coated Beads | 02-2207-00 | No Label Required | Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant. |
| Assay Buffer | 02-9953-00 | No Label Required | Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant. |

| Ingredient | Cat. No. | Label | |
|---|---|---|---|
| Standard Diluent | 02-0225-02 |  | **Warning.** May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Get medical advice/ attention if you feel unwell. Dispose of contents/ container to an approved waste disposal plant. |
| 10X Wash Buffer | 02-0001-03 |  | **Warning.** Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |

# Assay Preparation

## Reagent Preparation

1. Warm all reagents to room temperature (RT) prior to use.

1. Store the Detection Antibody away from light until ready to use.

2. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:

   - Pour all 3 bottles of 10X Wash Buffer (containing 50 mL each for 150 mL total) into a container capable of holding at least 2 L. Add 1.35 L of deionized water.
   - Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

   **Note:** 1X Wash Buffer may be filter sterilized (refer to Kit Storage).

3. Mix PD-1 Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥ 20 minutes until all beads are resuspended.

## Sample Preparation

**Preferred Method**

- Stack the filter plate on top of a 96-well receptacle plate.
- Place 250 µL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x *g*.

**Optional Method**

Centrifuge samples at > 13,000 x g for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

## Sample Dilution

- Dilute the clarified samples 1:2 using the Standard Diluent (for triplicates, transfer 200 µL of clarified sample to the sample preparation plate and add 200 µL Standard Diluent).
- 100 µL per well of 1:2 diluted Serum or Plasma should be used.
- If further sample dilution is required, samples can be diluted with the provided Standard Diluent.

## Initial Standard Stock Preparation

Reconstitute lyophilized standard in 250 µL of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes.

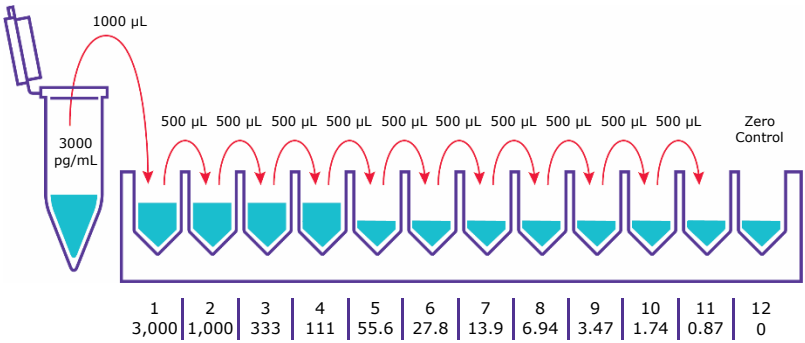
Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the PD-1 Standard in the vial.

Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 3,000 pg/mL in a 1.0 mL final volume.

## Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the 3,000 pg/mL Standard 1 for Standards 2 through 4 and 1:2 serial dilutions of Standard 5 through 11 to achieve a curve from 3,000 pg/mL to 0.87 pg/mL. Standard 12 is the Blank (Standard Diluent only).

Run the standards in triplicate.




**Note**: Pipette gently into reservoir wells to avoid creating bubbles.

1. Add 1,000 µL Standard Diluent to wells 2 through 4 of a 12-channel reservoir dilution plate.
2. Add 500 µL Standard Diluent to wells 5 through 12 of a 12-channel reservoir dilution plate.
3. Transfer 1,000 µL 3,000 pg/mL working stock (Standard 1) into well 1.
4. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

# Assay Procedure

## Target Capture

1. Pipette 100 µL per well of Standards or 1:2 diluted Samples to assay plate.
2. Retrieve the PD-1 Coated Bead vial from the rotator and transfer its full contents to 11.0 mL of supplied Assay Buffer. Rinse the bead vial with 0.55 mL of fresh Assay Buffer and ensure that all beads have been transferred from the original vial. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted PD-1 Coated Beads.
3. Using a multichannel pipette, add 100 µL per well of diluted PD-1 µL Coated Beads into the assay plate.
4. Seal the assay plate with clear adhesive plate seal, applying pressure to the seal to prevent leaking and cross-contamination.
5. Incubate for 2 hours at 25 °C on microplate incubator/shaker (Jitterbug™ setting #4).
6. A minimum of 10 minutes prior to the end of target capture incubation, prepare the PD-1 Detection Antibody working stock:

   Prepare 1X Detection Antibody by adding 250 µL of 20X Detection Antibody into 4,750 µL of Assay Buffer and filter the diluted Detection Antibody using the syringe with a 0.2 µm filter into a clean tube.
7. When incubation is complete, centrifuge the assay plate at 1,100 x *g* for 1 minute, place the plate on the washer magnet, and carefully remove clear adhesive plate seal to avoid splashing.

## Post-Capture Wash

Wash plate once with a plate washer (Bio-Tek® 405 TSUVS; Post Capture Wash (POSTCAP)). If using automation, please contact your technical service representative for the appropriate automation procedure.

## Detection Antibody Incubation

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Using a multichannel pipette, dispense 20 µL per well of PD-1 Detection Antibody using reverse pipetting without disturbing the bead pellets.
3. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate for 1 hour at 25 °C on microplate incubator/shaker set (Jitterbug™ setting #5). Ensure plate is protected from light during this incubation.
5. When incubation is complete, centrifuge at 1,100 x *g* for 1 minute then carefully remove the clear adhesive plate seal to avoid splashing.

## Post-Detection Wash

Wash the assay plate 4 times with wash buffer using the 4 cycle Pre-Transfer (4CYCPRE) program on the Bio-Tek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

## Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200 µL of wash buffer.
2. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
3. Place the plate on the microplate/incubator shaker (Jitterbug™ setting #3) for 2 minutes. Ensure plate is protected from light during this incubation.
4. Remove the plate from the shaker, and centrifuge at 1,100 x *g* for 1 minute. Carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

## Final Aspiration

Perform Final Aspiration using Bio-Tek® 405 TSUVS; Final Aspirate (FINASP). If using automation, please contact your technical service representative for the appropriate automation procedure.

## Elution

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet.
3. Seal assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate the plate for 10 minutes at 25 °C on microplate incubator/shaker (Jitterbug™ setting #5). Ensure plate is protected from light during this incubation.
5. When incubation is complete, centrifuge at 1,100 x *g* for 1 minute.

# Assay Reading

## To read on the SMCxPRO® or FemtoQuest™ System

1. Place the assay plate with Elution Buffer B onto the sphere mag plate and allow beads to form a tight pellet for 2 minutes.
2. Keeping the assay plate on the magnet, carefully remove the adhesive plate seal. Using a multichannel pipette, add 10 µL of Buffer D to center of wells containing Elution Buffer B. Use a fresh tip with each dispense.
3. Set a manual 12-channel pipette (1-20 µL) to 18 µL and put 12 tips onto the pipettor. Transfer 18 µL of neutralized eluate solution per well to corresponding wells of the SMC® Read Plate, placed over the included plate holder by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal the 384-well SMC® Read Plate with new clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 x *g*. Remove the seal, inspect SMC® Read Plate wells and remove bubbles if they are present.
5. Firmly seal the SMC® Read Plate with aluminum plate seal using the recommend plate roller.
6. Remove the plate holder from the sealed reading plate and load it onto the SMCxPRO® or FemtoQuest™ System. Start read.

   **Note:**

   For SMCxPRO® System: There is a warmup period of up to 30 minutes to wait for the SMC® Read Plate to be close to the internal instrument temperature. Once achieved the read will start automatically.

   For FemtoQuest™ System: The Immunoassay System will wait (up to 30 minutes) to allow SMC® Read Plate to equilibrate to internal instrument temperature. The 'Status' message, 'Waiting' will be displayed. Once the instrument is ready to read plate, status will change from 'Waiting' to 'Moving to Well' to 'Well Scanning'.

# SMC® Assay Overview

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 µL of Standard/1:2 diluted samples and 100 µL of Coated Beads to assay plate.
3. Seal and incubate for 2 hours at 25 °C on appropriate microplate incubator/shaker.


2 hours 25 °C

4. After capture incubation, centrifuge assay plate at 1,100 *x g* for 1 minute.
5. Perform Post-Capture Wash.
6. Remove from washer magnet and add 20 µL of Detection Antibody per well.
7. Seal assay plate and incubate for 1 hour at 25 °C on microplate incubator/shaker.


1 hour at 25 °C

8. Perform Post-Detection Wash.
9. Seal the assay plate and perform the post-detection shake for 2 minutes on microplate incubator/shaker.
10. Perform the Final Aspiration.
11. Remove from washer magnet and add 10 µL of Elution Buffer B to each well of assay plate.
12. Seal assay plate and incubate for 10 minutes at 25 °C on microplate incubator/shaker.


10 minutes at 25 °C

13. Add 10 µL of Buffer D to neutralize the eluted antibody.
14. Transfer 18 µL of neutralized eluate to SMC® Read Plate.
15. Seal SMC® Read Plate with aluminum adhesive plate seal for SMCxPRO® or FemtoQuest™ System.
16. Load on SMCxPRO® or FemtoQuest™ System.

# SMCxPRO® Assay Characteristics

## Sensitivity

Assay sensitivity measures the true limit of quantitation of an analyte and is often defined by the Lower Limit of Quantification (LLOQ). LLOQ is calculated as the lowest concentration that can achieve CVs of < 20% and the percent recovery of the standard point is still between 80%-120%. The LLOQ of PD-1 is 1.74 pg/mL. Please note that the published LLOQ is data generated during kit verification and can have minor variation between kit lots. For lot specific LLOQ data, please see the Certificate of Analysis.

## Precision

The assay variations of SMC® Human PD-1 Immunoassay kits were studied using fifteen normal serum and plasma samples run in triplicate by 3 different operators on 3 different days.

- Mean intra-assay variation was < 15%
- Mean inter-assay variation was < 20%

## Cross-Reactivity

Cross-reactivity to the following analytes were tested with the following results:

PD-L1 – < 0.1% Cross-reactivity

Specificity to the following species samples were tested with the follow results:

- Sprague-Dawley Rat – 4 out of 4 individuals quantifiable
- BALB/C Mouse – 4 out of 4 individuals quantifiable
- Cynomolgus Monkey – 4 out of 4 individuals quantifiable
- Rhesus Monkey – 4 out of 4 individuals quantifiable
- Canine (Beagle) – 4 out of 4 individuals quantifiable

**Note:** Assay has not been verified for the mentioned species.

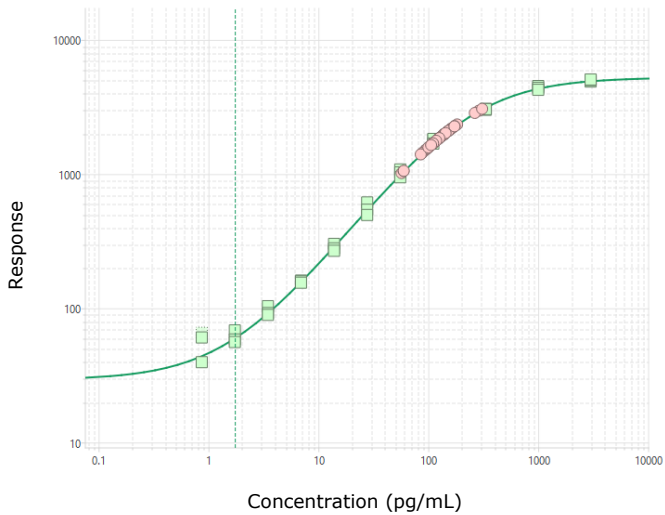
## Spike Recovery

The data represent mean percent recovery of three different concentrations of standard spiked into samples (n = 5 serum samples, 5 plasma samples)

| Sample ID | Serum Recovery | Plasma Recovery |
|---|---|---|
| Sample 1 | 88 | 53 |
| Sample 2 | 96 | 81 |
| Sample 3 | 93 | 97 |
| Sample 4 | 80 | 84 |
| Sample 5 | 109 | 100 |
| **Average** | **93** | **83** |

# Graph of Typical Reference Curve

Typical SMCxPRO® Human PD-1 Immunoassay Standard Curve, not to be used to calculate data.



# Troubleshooting

| Problem | Probable Cause | Solution |
|---|---|---|
| Background is too high | Background wells were contaminated | Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected. |
| | | Ensure reagents (including Wash Buffer) are not contaminated. |
| | | Insufficient washes—washer may need to be cleaned or reprogrammed. |
| | Plate was over-incubated | Confirm plate incubation times are as recommended, particularly for the Detection incubation. |
| Sample variability is high | Multichannel pipet may not be calibrated | Calibrate pipets. |
| | Plate washing was not uniform | Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well. |
| | Samples may have high particulate matter or other interfering substances | Samples should be filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision. |
| | Plate agitation was insufficient | Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing (See Jitterbug™ Shaker settings in Assay Best Practices). |
| | Cross-well contamination | Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x *g* for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed. |
| | | Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate. |

| Problem | Probable Cause | Solution |
|---|---|---|
| Beads are lost during the wash. | Plate washer needs optimization/cleaning | Contact Tech Support or local Specialist to schedule washer programming. Refer to user guide for cleaning procedure. |
| | Insufficiently primed washer | Washer should be primed with wash buffer prior to running the post capture wash protocol. |
| | Beads came in contact with water | Washer should be primed with Wash Buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles. |
| | Proper magnet was not used | Ensure that the SMC® magnetic plate shipped with the BioTek® 405 TSUVS Plate Washer was present on plate wash stage prior to running wash protocol. |
| Published LLoQ was not achieved | Improper dilution/reconstitution of the standard reference material | Confirm appropriate kit protocol was followed when preparing standard curve. |
| | | Check plate washer to confirm no beads were lost during washes and that plate contains < 2 µL following the post-capture and final aspiration protocols. |
| | | Ensure standards are prepared before starting capture incubation. |
| Microparticles do not resuspend into homogenous solution | Beads were not properly stored and may have been frozen | Labelled microparticles should be stored at 4 °C. If microparticles are frozen, they will not resuspend properly. |
| | Samples may be causing interference due to excess particulate matter | Samples should be properly processed prior to testing to remove particulate matter or lipids. |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 | Standard 8 | Standard 9 | Standard 10 | Standard 11 | Standard 12 |
| B | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 | Standard 8 | Standard 9 | Standard 10 | Standard 11 | Standard 12 |
| C | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 | Standard 8 | Standard 9 | Standard 10 | Standard 11 | Standard 12 |
| D | Sample 1 | Sample 1 | Sample 1 | Sample 2 | Sample 2 | Sample 2 | Sample 3 | Sample 3 | Sample 3 | Etc. | Etc. | Etc. |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

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"Product" means FemtoQuest™ Instrument, 95-0200-00, 70-0200-00.

"Commercial Product" means (i) any product intended for sale; (ii) any

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"Research Use" means any internal in vitro research use. Such research specifically excludes the following uses of whatever kind or nature:

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- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the manufacture or sale of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product to provide a service to any third party

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## Safety Data Sheets (SDS)

Safety Data Sheets are available on the product page at SigmaAldrich.com.

## Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.



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